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INFECTIOUS MULTIPLE DRUG RESISTANCE IN THE ENTEROBACTERIACEAE

ANNUAL PROGRESS REPORT

by

Stanley Falkow, Ph.D.

supported by

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The structural gene for plasmid-mediated ampicillin resistance resides upon ε 3.2 x 10^6 dalton transposable sequence, TnA, flanked by short inverted repeated sequences which accompany its insertion. TnA was transposed to a small 1.8 x 10^6 dalton plasmid to form the recombinant plasmid RSF1050. Random deletions were introduced into RSF1050 by a combination of *in vitro* nuclease treatments. The deletions were mapped by digestion with restriction endonucleases and electron microscope analysis of DNA heteroduplexes. Trans-

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position deficient derivatives were found to contain deletions which included either one of the inverted repeated sequences or the central portion of TnA. Complementation experiments suggest that the terminal inverted repetition and the central region of TnA play different essential roles in TnA transposition.

Over the past several years plasmids mediating resistance to ampicillin and other penicillin derivatives as well as tetracycline, kanamycin and chloramphenicol have been identified in strains of Haemophilus influenzae. We have shown by several independent methods that these resistance determinants are identical to the transposable antibiotic resistance genes of enteric species. During the past year penicillinase producing gonococci have been identified in U.S. Military personnel returning from duty in the Far East. Thus far, these men represent the only known pool of such organisms in the continental United States. We have determined that the penicillin resistant gonococcal isolates carry a plasmid which carries about 40% of the TnA sequence. Thus, the recent findings in both H. influenzae and N. gonorrhoeae suggest that direct or indirect extension of the resistance pool of enteric bacteria to other heretofore universally susceptible gram negative bacteria.

The discovery that antibiotic resistance genes are often part of a highly evolved discrete genetic element which can migrate from DNA molecule to DNA molecule has profound epidemiological implications. Plausible mechanisms exist by which pathogenic microorganisms, previously antibiotic sensitive, may be converted to stable antibiotic resistance by the direct extension of an R plasmid or by the simple addition of only a segment of DNA to a pre-existing indigenous plasmid pool.

Preface

Over the past few years we have examined a novel mechanism by which antibiotic resistance genes become disseminated from plasmid to plasmid. This year we particularly concentrated upon the basic biochemical mechanisms underlying the transposition of ampicillin resistance from plasmid to plasmid. As has so often been the case in our work, however, there were two unexpected clinical findings that were directly relevant to our work. One of these clinical findings, the isolation of β-lactamase producing gonococci was of unique military importance since it solely affected U.S. military personnel stationed in the Far East. As noted in this report our studies on the transposition of ampicillin resistance in enteric species and Haemophilus influenzae were extremely useful in working out the nature and possible origins of penicillin resistance in the gonococcus.

As in previous years, the students in my laboratory have made the most significant contributions to this research program. I wish to particularly thank Frederick Heffron, Ronald Gill and Patricia Bedinger, for their innovative work. The preliminary work reported here on gonococci is the work by Dr. L. P. Elwell and Marilyn Roberts.

Introduction

Plasmids are extrachromosomal DNA elements of bacteria which may be transmitted directly or indirectly among different species of bacteria. Plasmids are known to carry a variety of genes that may be of selective advantage to the host bacterial cell under certain selective environmental conditions. The best known example of bacterial plasmids are the R factors which carry genes determining resistance to antimicrobial agents. Indeed, resistance to all of the common antibiotics used therapuetically have been identified on R plasmids and individual R plasmids may carry resistance for as many as 10 distinct antibiotics. Although R factors have been known since 1958, until recently little has been known about the mechanisms involved in their formation or the origin of their carried resistance genes.

Over the past two years a number of plasmid-mediated antibiotic resistance genes have been found to reside as part of discrete DNA sequences capable of transposition from replicon to replicon independently of the recA functions of the host bacterial cell (Hedges and Jacob, 1974; Kopecko and Cohen, 1975; Heffron, et. al., 1975a,b; Berg, et. al., 1975; Kleckner et. al., 1975). One such transposible sequence mediating ampicillin, TnA, is 4.8 kilobases (Kb) in size (about 3.2×10^6 daltons) and is flanked by short inverted-repeated sequences of about 140 base pairs (Kopecko and Cohen, 1975; Heffron, et. al., 1975a,b; Rubens, et. al., 1976; Bennett and Richmond, 1976). Insertion of TnA into a plasmid appears to depend upon recognition of a specific, but fairly common, nucleotide sequence (Heffron, et. al., 1975b; Rubens, et. al., 1976). Moreover, TnA may be inserted into DNA in either of two orientations and is mutagenic when inserted within a structural gene and polar (at least in one orientation) when insertion occurs within an operon (Rubens, et. al., 1976). In many ways TnA resembles the transposible IS sequences of E. coli in its genetic effects

(Jordan et. al., 1968; Shapiro, 1969; Hirsch et. al., 1968; Malamy et. al., 1972).

In the simplest terms, the transposition of TnA permits the ampicillin resistance gene to travel from R factor to R factor or even from R factor to chromosome. Of course this finding, which involves not only ampicillin resistance but kanamycin resistance (Berg, et. al., 1975) tetracycline resistance (Kleckner, et. al., 1975), chloramphenicol resistance (Gottesman and Rosner, 1975) and trimethoprim-streptomycin resistance (Barth, et. al., 1976) is of considerable biological interest but, additionally, is of considerable importance in the epidemiology of certain infectious diseases. Thus, for example, we can now understand why so many different plasmids of apparently diverse origin carry the identical β-lactamase protein (Heffron, et. al., 1975a; Matthew and Hedges, 1976). In the broader sense the ability of drug resistance genes to be transposed between DNA molecules independent of bacterial rec functions surely helps to explain the rapid evolution of R plasmids which possess varied permutations of antibiotic resistance genes. We suppose that the evolutionary "success" of R plasmids in modern society probably speaks most eloquently for the efficiency of transposition in nature. Nonetheless, the precise mechanism by which elements such as TnA were evolved as well as their mechanism of transposition still remains unclear. In order to gain a better insight into the nature of TnA we invested a considerable amount of effort over the past contract period in the isolation and characterization of TnA deletions in an attempt to define better the structural and biochemical basis of transposition. During the course of these investigations we further showed that the ubiquitous TnA element had gained entry to Neisseria gonorrhoeae and that this TnA segment in the gonococcus resided upon a plasmid closely related to a Haemophilus influenzae plasmid described in our last research proposal.

Results

A. Deletions affecting the transposition of TnA

As noted in last years report, if two plasmids are co-resident in the same cell it is possible to demonstrate transposition of TnA from one plasmid to the other. The plasmid RSF1050 was derived in this way by transposition of the 3.2×10^6 dalton TnA sequence from a large conjugative R plasmid to the 1.8×10^6 dalton ColE1 derivative pMB8. The resulting 5×10^6 dalton RSF1050 plasmid provided an excellent model in which to analyze deletions of TnA since TnA comprised better than 50% of the total plasmid sequences. Random deletions were introduced into RSF1050 by an *in vitro* procedure similar to that recently described by Carbon et. al. (1975). Covalently closed circular (CCC) RSF1050 DNA was treated with pancreatic DNAse in the presence of Mg^{++} to introduce, on average, 0.3 single-strand breaks per molecule. After separation of the nicked (OC) molecules from the unnicked CCC molecules by dye-isopycnic centrifugation, the OC DNA was treated with *E. coli* exonuclease III to introduce a small (on average 600 base pair) single-stranded gap. The gapped DNA was in turn treated with S1 endonuclease to remove the short single-stranded sequence and leave linear permuted plasmid DNA. The linear DNA was purified by sedimentation through a neutral sucrose gradient and used to transform (Cohen, Chang, and Hsu, 1972) $CaCl_2$-treated *E. coli* C600 containing an F Km plasmid (F Km is the classical F factor carrying resistance to kanamycin resistance). The transformants were selected for ampicillin resistance (Ap^r). Thus, transformant cells were expected to contain F Km as well as various deletion mutants of RSF1050. In theory the deletions would be random although the selection procedure dictated that the structural gene for β-lactamase and replication be preserved in any RSF1050 plasmid. The question was how to detect transposition deficient mutants of RSF1050.

RSF1050 possesses the ColE1 replication machinery and as such has a strict

dependence upon DNA polymerase I (Kingsbury and Helinski, 1970). We exploited this property in our search for mutants that were transposition deficient. Individual Ap^r transformant clones of C600 (F Km + RSF1050) were mated with a nalidixic acid resistant [Nal^r] DNA polymerase I deficient [pol 1^-] derivative of E. coli called SF800. The mating mixtures were plated on a medium selective for Ap^r Km^r [Nal^r] colonies. Since RSF1050 cannot replicate in the SF800 strain any Ap^r Km^r [Nal^r] colonies growing on the selective medium represented cells which had received F Km carrying a transposed TnA segment. The failure to detect Ap^r Km^r [Nal^r] SF800 colonies in any given mating suggested that the donor C600 (F Km + RSF1050) culture harbored a transposition deficient mutant. In point of fact, 25 transposition-deficient RSF1050 derivatives were isolated in this fashion. Parenthetically, it should be noted that this same basic rationale was reported in last years annual report as a means to 'mark' plasmids that synthesize enterotoxin, Ent plasmids, with antibiotic resistance genes. Using the four restriction endonucleases BamH, EcoRI, HincII and HaeII we have determined a map of RSF1050 (Top line, Fig. 1) by sequentially digesting plasmid DNA with pairs of enzymes and examining the resultant linear fragments by agarose gel electrophoresis. As anticipated, treatment of DNA from the RSF1050 transposition deficient mutants with these same enzymes showed fragment patterns which differed from the parental plasmid and so permitted the mapping of many of the deletions affecting transposition. The mapping of the deletion mutants was further facilitated by electron microscope heteroduplex analysis (Fig. 2). The results obtained from mapping several of the deletion mutants is shown in the lower part of Fig. 1.

Some of the transposition deficient derivatives of RSF1050 were deleted for little more than one of the inverted-repeated sequences that flank TnA. This finding substantially re-enforces the importance of these terminal segments for

transposition, a conclusion that has often been inferred (Kopecko and Cohen, 1975; Heffron, et. al., 1975a,b; Kleckner, et. al., 1975; Rubens, et. al., 1976). It may be noted that all of the deletion mutants affecting the IR sequences of RSF1050 have encompassed only the single IR on the "left" hand and of the TnA map (see Fig. 1). This is likely a reflection of the fact that the genetic selection employed to isolate the deleted derivatives of RSF1050 dictated that the structural gene for ampicillin resistance and the origin of replication be preserved. It may also be noted that all of the transposition deficient derivatives of RSF1050 had deletions which started within the TnA sequence but many of them included a large portion of other RSF1050 sequences (including colicin immunity). This finding suggests that a considerable portion of the RSF1050 genome can be deleted without affecting plasmid-maintenance and replication.

Certainly the most interesting finding arising from the analysis of RSF1050 deletion mutants was that the loss of only a small portion of the central region of TnA lead to a transposition deficient phenotype. This observation suggested that one or more essential functions were encoded in this region. In order to pursue the possibility that essential transposition functions are encoded within the central region of TnA, complementation studies were performed. Recombination deficient (recA), E. coli strains were constructed containing three coexisting plasmids: F Km, one of the RSF1050 deletion mutants and a plasmid RSF103 (RSF103 is unrelated to RSF1050 but carries a TnA segment deleted for part of the β-lactamase gene). The basic premise was that functions deleted in the RSF1050 transposition negative mutants might be complemented in trans by a protein produced by RSF103. We supposed this would permit transposition of the deleted TnA segment of the RSF1050 derivative to F Km. In turn, the F Km containing the inserted, deleted TnA segment would be detected by transfer to the [Nal^r] [pol 1^-] E. coli SF800 strain described earlier. The results of the complementation experiments demonstrated a definite difference between dele-

tions which included the terminal IR sequence and those encompassing only internal sequences. Deletions including the terminal sequence could not be complemented and did not transpose at a detectable frequency. Deletions confined to the central region of TnA were complemented and transposed to F Km at a frequency of about 0.18 - 0.24 of normal. The most attractive interpretation of the complementation data is that one (or more likely both) terminal sequences of TnA serve a structural role in the transposition process while the central region encodes for a diffusable substance, presumably a protein(s) which is required for transposition.

B. The R plasmids of H. influenzae

During the time that we were investigating the basic mechanisms of TnA transposition in enteric species we became aware of an increasingly serious and widespread problem of disease due to ampicillin resistant H. influenzae (see, for example, Tomeh, et. al., 1974). Since it was reported that these H. influenzae isolates produced a β-lactamase very much like that produced by enteric species, it seemed reasonable to examime these isolates for the presence of R plasmids. Consequently, some eighteen independently isolated ampicillin resistant, β-lactamase producing H. influenzae type b isolates from meningitis, epiglottitis and normal patients have been examined for the presence of R plasmids. Sixteen of the isolates contain a 30×10^6 dalton plasmid while two contained a 3.5×10^6 dalton plasmid. Typical ampicillin sensitive H. influenzae do not usually contain plasmid DNA. (Of 180 isolates examined only 5 contained plasmid species which were found to be unrelated to the R plasmid DNA by DNA-DNA hybridization). Transformation of sensitive H. influenzae to ampicillin resistance could be accomplished with purified plasmid DNA showing conclusively that the structural gene for β-lactamase resided upon these plasmid species.

The question then before us was whether these R plasmids of H. influenzae carried the TnA sequence that we had identified in enteric species. By both DNA-DNA hybridization studies as well as electron microscope heteroduplex analysis we could, in fact, show that the 30 x 10^6 dalton plasmid contained the complete TnA sequence while the 3.5 x 10^6 dalton plasmid contained but 40% of TnA. In the latter instance the TnA includes one of the terminal inverted-repeated sequences and includes the single BamHI site of TnA (see Fig. 1). Subsequent studies revealed that both of these H. influenzae R plasmids possessed an overall guanine + cytosine (G + C) content of 39%, very much like that of the H. influenzae chromosome and unlike that of most known enteric R plasmids. Since R plasmids often possess GC conents similar to their species of origin one might suppose that this suggests that transposition of TnA to some indigenous H. influenzae plasmid had occurred. There is, however, no definitive data to differentiate between the alternatives that the plasmid in H. influenzae represent a direct extension of existing R plasmids from enteric species or if there has been transposition of TnA from an enteric R plasmid to an indigenous Hamophilus plasmid. The full details of this work has been already published (Elwell, et. al., 1975; De Graaff, et. al., 1976).

More recently, R plasmids of H. influenzae encoding for tetracycline (Dang Van, et. al., 1975) as well as tetracycline and chloramphenicol resistance (Klingerin, Van Embden and Dessen-Kroon, in press) have been isolated. We have, in collaboration with M. H. Richmond, University of Bristol, England and J. Saunders (University of Liverpool, England) showed that 1) these resistances are plasmid-mediated, 2) these plasmids contain identical translocation sequences as those found in enteric species and 3) that the ampicillin, tetracycline and tetracycline-chloramphenicol R plasmids of H. influenzae all contain a common core of DNA sequences in common. Indeed, electron microscope heteroduplex

analysis reveals that these plasmids are essentially identical except for the inserted antibiotics resistance sequences. While this finding is most in accord with the idea that transposition of resistance genes to an indigenous plasmid has occurred, it is still a hypothetical possibility and by no means proved. It does seem fair to say, however, that these plasmids in H. influenzae represent the direct or indirect extension of the R plasmid pool of enteric species.

C. R plasmids of the Gonococcus

In our research proposal submitted in January 1976 for the contract year 1 June 1976 - 30 May 1977 we stated "...one may anticipate that the spread of antibiotic resistance to all gram negative species is inevitable and that, for example, the spectre of plasmid-mediated penicillin resistant N. gonorrhoeae will only be a question of time". Despite this predictive statement, we were surprised when Dr. Clyde Thornsberry, Center for Disease Control, Atlanta, Georgia contacted us in the early summer of 1976 to inform us that numerous isolations of penicillin-resistant, β-lactamase producing N. gonorrhoeae were being encountered from U.S. Military personnel returning from the Far East (see Ashford, et. al., 1976). We were also independently notified by Canadian Officials that returning members of their Armed Forces were also contracting these infections. Shortly, thereafter, a report was published from London, England (Phillips, 1976) showing the presence of a β-lactamase producing gonococcus from the vagina of a woman suffering from pelvic inflammatory disease. However, all cases of resistant gonococci isolated, thus far, in the continental U.S.A. have been traced directly or indirectly to U.S. military personnel.

Fortunately, we had recently developed an agarose gel electrophoresis technique which permits the rapid identification and partial characterization

of bacterial plasmids. The application of this method to the gonococcal isolates revealed that the β-lactamase gene of the gonococci from returning service men and their sexual contacts was mediated by a plasmid some 4.4×10^6 daltons in mass. We were able to isolate this plasmid in pure form and are in the process of characterizing its properties. While our studies of gonococci are specifically funded from other sources, one aspect of the work was revelant to our current contract mission and contract personnel and funds have been employed for this one aspect of the gonococcal work. Naturally, this aspect was whether the gonococcal plasmids carried all or part of TnA. DNA hybridization studies have, indeed, showed that the gonococcal plasmids carry 40% of TnA. But perhaps the most intriguing part of our contract related studies of the gonocojcal plasmids may be seen in Table 1. The small 3.5×10^6 dalton H. influenzae plasmid and the gonococcal plasmids are very highly related suggesting a common origin. One certainly cannot say that this finding indicates a direct extension of the small R plasmid of H. influenzae to N. gonorrhoeae. Rather because of size differences as well as the independent nature of the London isolate (see Table 1) it seems more likely that there is a common source or mechanism by which R plasmids are now entering non-enteric gram negative species. It is a most fascinating epidemiological finding. At a more practical level it is apparent that the use of the standard 4.8 megaunit-penicillin treatment regimen in U.S. Military personnel is in danger. The alternative, 2 or 4g IM spectinomycin has proved to be an effective alternative (Ashford, et. al., 1976; CDC Advisory 1976). But it should be pointed out that spontaneous mutation to spectinomycin is by no means uncommon (see Falkow, et. al., 1976). Moreover, the rapid succession of R plasmids in H. influenzae documented by our contract studies provides a disquieting precedence for the future of antimicrobial therapy of gonococcal infections.

Discussion

In last years annual report we considered at some length the epidemiological implications of transposable antibiotic resistant determinants. Transposition provides a reasonable explanation for the accumulation of resistance genes by plasmids. The evolutionary flexibility that attends transposition is of enormous selective advantage. There are probably two major new points that have emerged from this years contract work. The first of this point speaks to the basic mechanisms underlying the transposition phenomenon as such. Direct and inverted DNA repetition have been shown to flank all transposable antibiotic resistance genes studied thus far. In several instances these flanking sequences have been found to be known IS sequences described in bacterial, bacteriophage and plasmid DNA. For example, the transposable tetracycline resistance determinants has been shown to be bracketed by inverted repeated IS3 sequences (Patashne and Cohen, 1975). In view of the ability of IS sequences to themselves be transposed, it has been suggested that DNA repetitions flanking antibiotic resistance genes are responsible for the mobilization of resistance genes from plasmid to plasmid (Kopecko and Cohen, 1975; Berg, et. al., 1975; Rubens, et. al., 1976). TnA resembles other transposable antibiotic resistance genes is being bracketed by an inverted DNA repetition, But the short 140 base pair inverted repetitions flanking TnA are not known to correspond to any characterized IS sequence nor does it seem likely that these short sequences can, like IS3, in themselves act as transposable elements. Moreover, our analysis of TnA deletions show that the DNA included between the short terminal repeated sequences appears to be much more than just so much "excess baggage" which happens to include the selectively advantageous structural gene encoding for β-lactamase. Rather the transposition deficient derivatives of RSF1050 contain deletions which map across a sizable portion of the central

region of TnA indicating that far more than the terminal sequences are required for transposition. Certainly the results we obtained are in agreement with the idea that within the terminal region of TnA are sequences which by analogy to temperate bacteriophages, serve as recognition sites for an enzyme(s) which controls all or part of the excision and integration aspects of the transposition process. Our results would further suggest that one or more of these transposition enzymes may be encoded in the central portion of TnA. Thus for, TnA at least, transposition appears to be a highly evolved specific process.

The second major point which we feel seems to be consistent with our data is based on speculation from our combined epidemiological and laboratory work on the distribution of TnA. This speculation stated in its most simplistic terms in that there has been the selection for some class of R plasmids of very broad host range which is capable of breaching genetic barriers that heretofore have remained impenetrable. One class of R factors, the P group, arising from Pseudomonas aeruginosa, does in fact, seem to correspond to such an agent (reviewed in Falkow, 1975). Nonetheless, it does not seem that the direct extension of a broad host range plasmid such as the P group is the answer. For example, the 30×10^6 dalton plasmid encoding for resistance common in H. influenzae is 39% G + C, while P plasmids (and other plasmids of the pseudomonads) possess G + C contents of 60% or greater. Indeed, the 30×10^6 dalton Haemophilus plasmid do not show sequence homology with any of the common enteric R plasmids. As we pointed out earlier it is, of course, conceivable that an enteric R plasmid has penetrated a remote species such as Haemophilus and 'donated' its resistance gene(s) by transposition to an indigenous plasmid. This is an attractive hypothesis but there is no experimental evidence to support this contention. Nor, does such a hypothesis fit with the finding that some plasmids of H. influenzae are small non-selftransferable plasmids or that the recently described plasmids of gonococci are also non-selftransferable and

while related to the small Haemophilus plasmid not by any means identical. Also, why in the small plasmids of both Haemophilus and Neisseria do we find that the complete TnA segement is absent. Rather, we find that only the comparable piece of TnA encompassing the 'right hand' inverted repeat and including the single BamHI site is present. And the Haemophilus plasmids and the gonococcal plasmids are 39% G+C and not associated with a transfer factor that might have been responsible for their mobilization. Does this suggest that there is a pool of non-selftransmissible plasmids that directly gain entry into diverse microbial species by some other mechanism, perhaps transformation? While we cannot answer these questions at present it is a fascinating epidemiological puzzle that has already had profound medical and economic impact. Continued and more intensive surveillance of resistance in universally susceptible species seems warrented.

Despite our intense interest in antibiotic resistance we intend to turn our research interests, at least under this contract, away from resistance to other more immediate problems. Specifically, we intend to apply our experience gained in the study of the molecular biology and transposition of antibiotic plasmids to those analogous agents which are important in diarrheal disease. This new direction of our efforts will be detailed in our next research proposal It seems unlikely, however, that we will be able to sharply demarcate the boundries between R plasmids and those plasmids which directly contribute to the pathogenesis of diarrheal diease. This has become all too apparent since we have recently isolated several plasmids which encode for resistance as well as a heat labile enterotoxin.

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Table 1

Relationship of *H. influenzae* Plasmid RSF0885
To R plasmid of *N. gonorrhoeae*

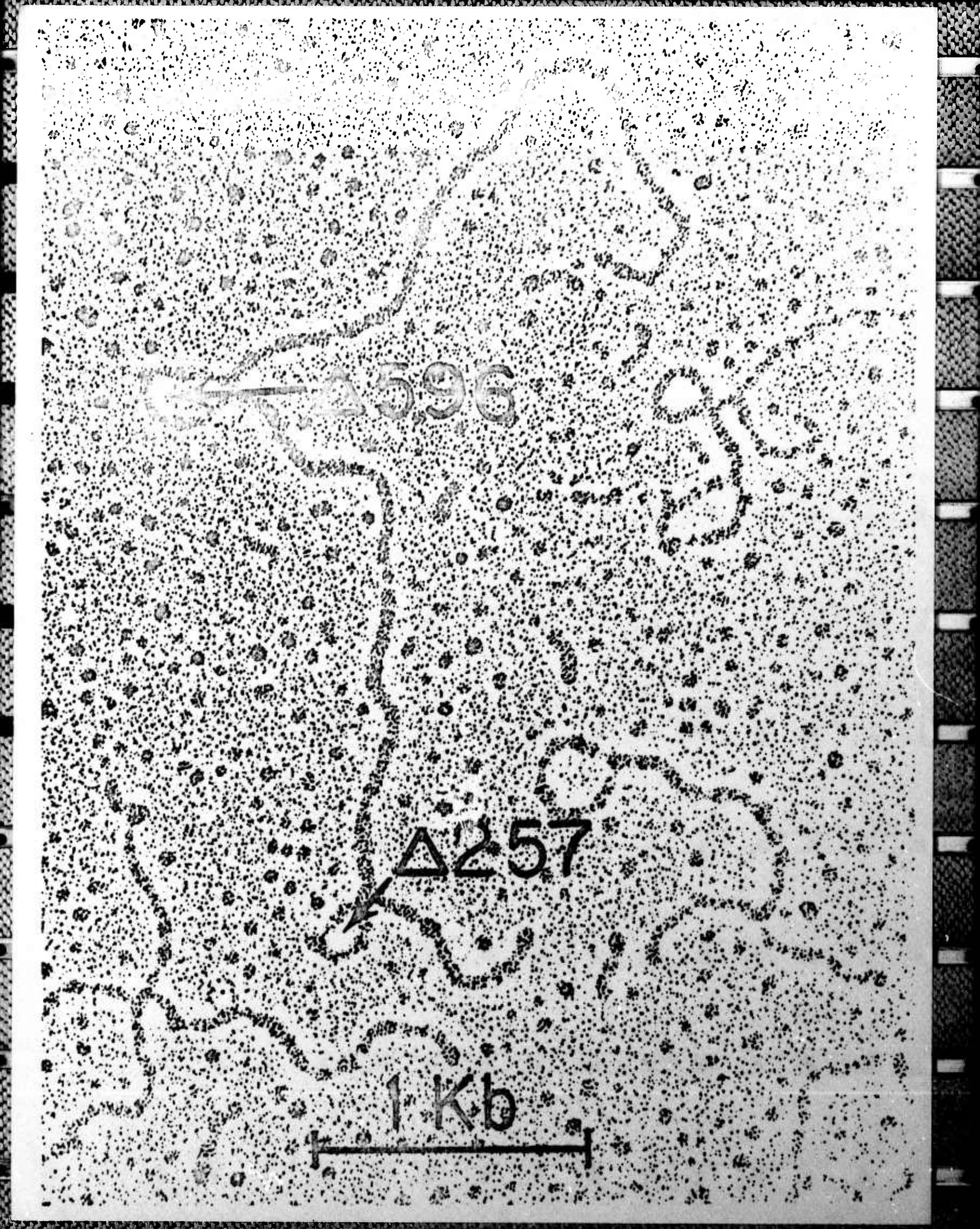
Plasmid	Source	Mol Wgt x 10^6 dalton	% G + C	% Relative DNA Sequence Homology with RSF0885 DNA
RSF0885[a]	*H. influenzae*	3.8	40	100
RSF0062[b]	*N. gonorrhoeae*	2.6	50	<1
RSF0045[b]	*N. gonorrhoeae*	24.5	50	1.2
RSF0066[c]	*N. gonorrhoeae*	4.4	40	91
RSF0068[d]	*N. gonorrhoeae*	3.3	40	64

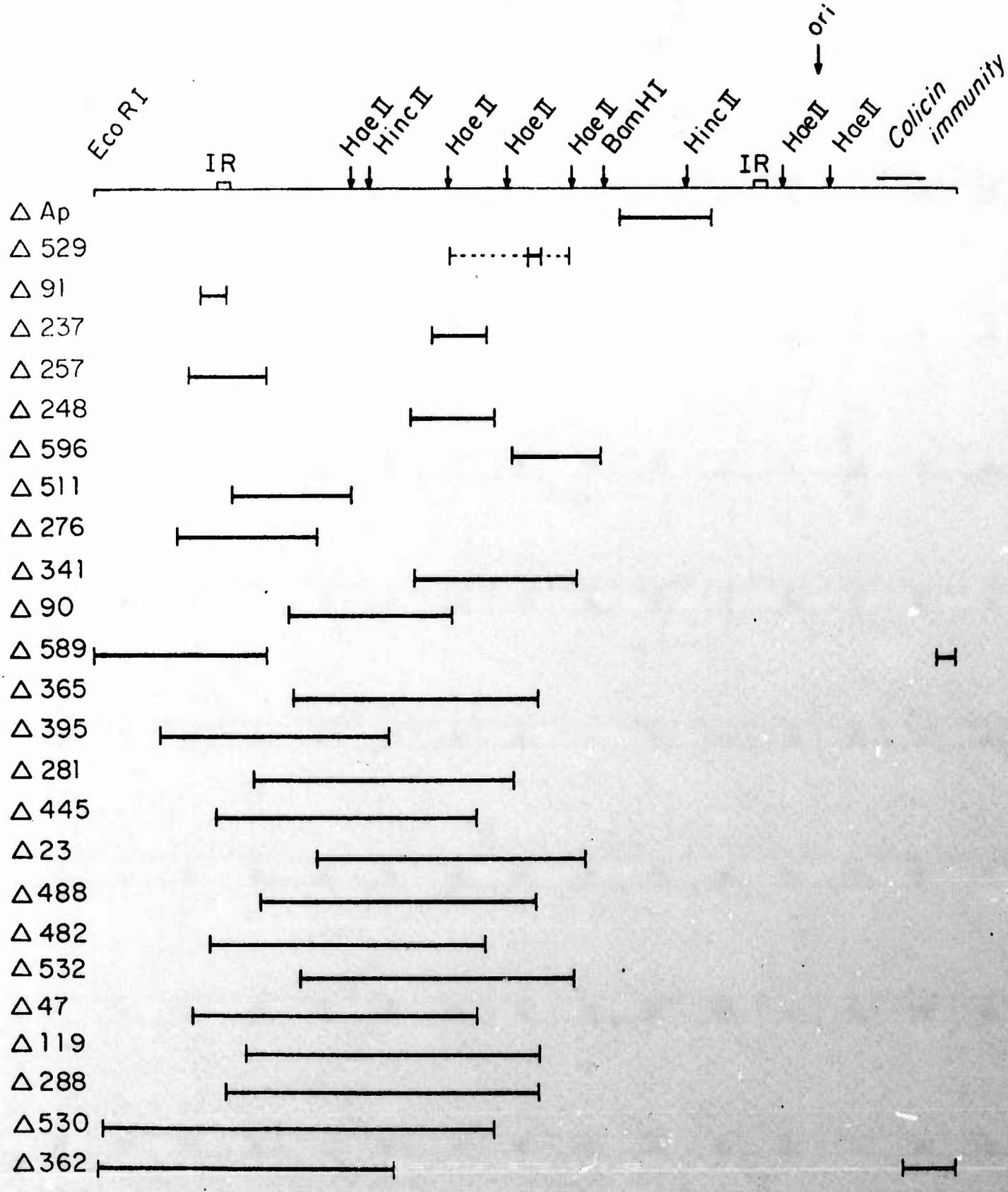
[a]Plasmid determining ampicillin resistance (de Graaff, et. al., 1976)

[b]Plasmids RSF0062 and RSF0045 are indigenous plasmids of gonococci commonly found in penicillin sensitive strains

[c]Plasmid determining penicillin resistance isolated from U.S. serviceman returning from Far East

[d]Plasmid determining penicillin resistance isolated from woman in London, England


Δ596
Δ257
1 Kb


ori
Eco RI
IR
Hae II
Hinc II
Hae II
Hae II
Hae II
Bam HI
Hinc II
IR
Hae II
Hae II
Colicin immunity
Δ Ap
Δ 529
Δ 91
Δ 237
Δ 257
Δ 248
Δ 596
Δ 511
Δ 276
Δ 341
Δ 90
Δ 589
Δ 365
Δ 395
Δ 281
Δ 445
Δ 23
Δ 488
Δ 482
Δ 532
Δ 47
Δ 119
Δ 288
Δ 530
Δ 362

Legend to Figure 1

Deletions affecting transposition of TnA. The upper line drawing represents EcoRI cleaved RSF1050. This plasmid is 7.4 kb in length and specifies colicin immunity and ampicillin resistance. The two bars labelled IR represents the short 140 base pair inverted repeated sequences which flank TnA and accompany its insertion. Ori, designates the origin of replication. Sites cleaved by the restriction enzymes EcoRI, BamHI, HincII and HaeIII are represented by arrows and are accurate to within ± 100 base pairs. Deletions which affect transposition are represented by solid bars for the DNA sequence that has been deleted. The deletion Δ529 is only 60 base pairs and has been determined to lie some where in the dotted region. The ΔAp deletion involves all or part of the structural gene for β-lactamase carried by TnA. The location of the structural gene for colicin immunity was inferred from the observation that RSF1050 deletion mutant Δ362 possessed a colicin E1 sensitive phenotype whereas mutant Δ589 and all other deletion mutants possessed the colicin immune phenotype.

Legend to Figure 2

Visualization of transposition-deficient deletions. The DNA of two EcoRI cleaved RSF1050 transposition-deficient mutants are shown heteroduplexed against each other. The deletion Δ257 deletes one of the inverted repeat sequences while the deletion near the center of the molecule Δ596 is immediately adjacent to the BamHI site and the structural gene for β-lactamase.

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